RESEARCH COMMUNICATION

The *ACE* Gene Polymorphism is Associated with the Incidence of Gastric Cancer among *H. pylori* Seropositive Subjects with Atrophic Gastritis

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Abstract

Studies of the angiotensin converting enzyme (*ACE*) *I/D* polymorphism have provided evidence that the *D/D* genotype is associated with gastric tumor progression and numbers of lymph node metastases, but not with the overall risk of gastric cancer. The highest levels of circulating and tissue ACE activity were found in carriers of the *D/D* genotype. Here, we further investigated the association using 454 Japanese subjects undergoing a health checkup and 202 gastric cancer patients. The *ACE* polymorphism was not found to be linked with *H. pylori* seropositivity or gastric atrophy. However, among *H. pylori* seropositive subjects with atrophy, those with the *I/D* genotype had an increased risk of gastric cancer (OR=1.59; 95% CI, 1.02-2.48). We also established that the polymorphism did not lower the age at diagnosis of gastric cancer. Confirmation of the association between *ACE* polymorphisms and development of gastric cancer requires much larger studies, and the biological role also needs to be fully elucidated.

Key Words: *ACE* polymorphisms - gastric cancer risk

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Introduction

In 2000, gastric cancer was the second most frequent cause of deaths from neoplasia and the fourth most common cancer in the world, with an estimated 650,000 deaths and 880,000 new cases per year, almost two-thirds of which occurred in developing countries (Stewart et al., 2003). In 2001, gastric cancer caused 50,000 deaths in Japan, and was the second most frequent cause of cancer mortality (Ministry of Health, Labor and Welfare, 2003). Gastric carcinogenesis is a multistep process in which genetic and environmental factors interact with each other (Dunbier et al.,2001; Correa, 1992; Tahara 1993; Stadtlander et al., 1999; Neugut, 1996). *Helicobacter pylori* (*H. pylori*) is considered to play a specific role in development of the atrophic gastritis that represents the most recognized pathway in multistep intestinal-type gastric carcinogenesis (Covacci et al.,1999; Crabtree et al.,1991; Kuipers et al., 1995).

Previous studies suggest that combinations of host genetic and bacterial virulence factors determine the severity of gastric damage and the eventual clinical outcome of *H. pylori* infection (El-Omar et al., 2000; El-Omar et al., 2001; El-Omar et al., 2003; Machado et al., 2001; Machado et al., 2003). Alterations in various genes, including oncogenes, tumor-suppressor genes, DNA repair genes, cell-cycle-related genes and cell-adhesion-related genes, have been implicated in the course of gastric carcinogenesis (Yasui et al., 2000; Werner et al., 2001; Gonzalez et al., 2002).

Angiotensin I-converting enzyme (ACE) is a type I cell surface zinc metallopeptidase, expressed by many cell types of various organs and tissue types (Bauvois, 2004) which generates Angiotensin II (AngII), the major effector in the renin-angiotensin system (RAS). Recent studies have revealed local expression of several components of RAS in various cancer cells and tissues, including brain, pancreatic and gastric cancer (Juillerat-Jeanneret et al., 2004; Fujimoto et al., 2001; Röcken et al., 2005).

A polymorphism in the human ACE gene has been reported, in which there is an insertion (I) or deletion (D) of a 287-base pair Alu-reptitive sequence in intron 16 (Rigat et al., 1992). The highest level of circulating and tissue ACE activity has been found in carriers of the D/D genotype (Rigat et al., 1990). Ang II receptors, primarily of Ang II type I receptor (AT1R) subtype, are expressed on tumor and

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endothelial cells, and are upregulated in many cancer tissues (Deshayes et al., 2005). It is established that AT1R induces cell proliferation in a variety of cellular models, including human cancer cells, by activating various intracellular cascades involving protein kinases (Uemura et al., 2003; Arrieta et al., 2005; Fujimoto et al., 2001; Muscella et al., 2002; Greco et al., 2003).

A previous investigation provided evidence that breast cancer risk is reduced in women possessing an ACE low activity genotype (Koh et al., 2003). Furthermore, a study of the ACE polymorphism in gastric cancer indicated that the D/D genotype is associated with tumor progression and the number of lymph node metastases but not with risk of tumour development (Röcken et al., 2005). To our knowledge, there there have been no other studies on links between the ACE polymorphism and risk of gastric cancer. The present study was therefore performed with the aim of evaluating this question, taking into account the prevalence of atrophic gastritis and *H. pylori* infection.

Materials and Methods

Study Subjects

Detailed information on the characteristics of the healthy control and gastric cancer patients in this study has been published in our previous papers. Briefly, the control group comprised 454 health checkup examinees (126 males and 328 females) aged 35 to 85 years with no history of cancer who attended a health checkup program supported by the Nagoya Municipal Government in August and September, 2000 (Katsuda et al., 2003). The case group consisted of 202 patients (134 males and 68 females) aged 33 to 94 years with a pathologically confirmed diagnosis of gastric adenocarcinoma who underwent tumor resection in different hospitals affiliated with Nagoya University (Goto et al., 2005). Informed consent was obtained from all subjects. Approval for the study was given by the relevant ethical committees.

Tests for H. pylori Antibodies and Pepsinogens

Anti-*H. pylori* IgG antibody tests, high-molecular-weight campylobacter-associated-protein (HM-CAP) ELISA (Enteric Products Inc., Westbury, NY) and HM-CAP with antigens extracted from clinically isolated Japanese H. pylori strains (J-HM-CAP) ELISA (Kyowa Medex, Tokyo, Japan), were used for the identification of *H. pylori*-infected participants. An ELISA value of 2.3 or over was regarded as positive for both tests. The infection was confirmed in all gastric cancer cases by culture and bacteriological tests (Gram-negative, oxidase, catalase, and urease testñpositive spiral, curved rods) using biopsy specimens before gastric resection. Pepsinogens I and II (PG I and PG II) in plasma were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). Gastric atrophy was defined as PG I< 70 ng/ml and PG I/PG II ratio <3. These parameters for atrophy are in wide use in Japan and have been validated against histological confirmatory studies.

ACE Genotyping

DNA was extracted from the buffy coat fraction with the Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc.,Valencia, CA, USA). The primers were F: 5-GCCCTGCAGGTGTCTGCA, R: 5-GCTCTCCCCGCCT TGTC TC. Genomic DNA was applied in a volume of 25 µl with 0.12 mM dNTPs, 25 pmol of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer Corp., Foster City, CA, USA), and 2.5 µl 10 x PCR buffer including 15 mM $MgCl_2$. The PCR was performed with initial denaturation at 95˚C for 10 minutes, followed by 30 cycles of denaturation at 95˚C for 1 minute, annealing at 60˚C for 1 minute and extension at 72˚C for 1 minute. Final extension was at 72˚C for 5 minutes. Amplified ACE gene fragments were separated on 2% agarose gel and visualized by ethidium bromide staining. D and I alleles were identified by the presence of 314 bp and 592 bp, respectively.

Statistical Analysis

Odds ratios (ORs) adjusted for sex and age with 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis. ACE polymorphism was tested using the Hardy-Weinberg equilibrium. We hypothesized the ACE genotype may influence the age at diagnosis of gastric cancer. To confirm this hypothesis, we estimated the difference of the age at diagnosis according to ACE polymorphism by a Kaplan-Meier method. The comparison between genotype groups was made using a Wilcoxon test. Calculations were performed using the computer program STATA v. 8 (STATA Corp, College Station, TX, USA).

Results

The genotype distribution of the control group fit the

Table 1. The Distribution of the *ACE* Genotype in Subjects as a Whole and in *H. pylori* Seropositive Subjects with Gastric Atrophy (GA)

	Whole subjects				Seropositive subjects with GA			
Genotype	Cases	Controls	OR*	95%CI	Cases	Controls	OR*	95%CI
I/I	76 (37.6%)	209 (46.0%)	1.00	Reference	64 (35.8%)	121 (44.7%)	1.00	Reference
I/D	98 (48.5%)	189 (41.6%)	1.38	0.93-2.05	89 (49.7%)	111 (41.0%)	1.59	1.02-2.48
D/D	28 (13.9%)	56 (12.3%)	1.26	0.71-2.24	26 (14.5%)	39 (14.4%)	1.22	0.66-2.29
I/D + D/D	126 (62.4%)	242 (53.3%)	1.35	0.93-1.96	115 (64.3%)	150 (55.4%)	1.49	0.98-2.26

* Adjusted for age and sex by unconditional logistic regression.

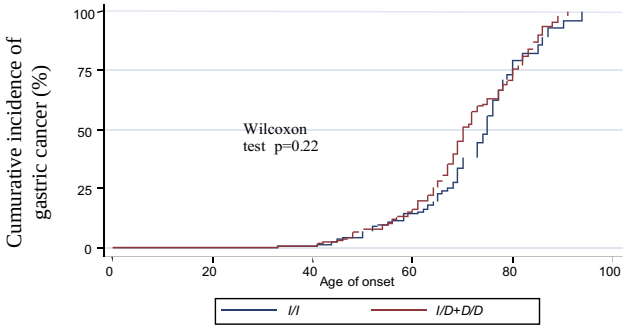

Figure 1. Association between ACE I/D polymorphism and the Age at Diagnosis of Gastric Cancer. The distributions of age at diagnosis according to ACE polymorphism were estimated by a Kaplan- Meier method and compared using a Wilcoxon test.

Hardy-Weinberg equilibrium (χ^2=1.67, P=0.20). The clinical characteristics of the cases and the controls were reported previously (Goto et al., 2005). Among the healthy controls, the ACE polymorphism was not associated with *H. pylori* seropositivity and gastric atrophy. The ORs of the I/D and D/D for *H. pylori* seropositivity relative to I/I were 1.12 (95% CI, 0.74-1.69) and 1.24 (95% CI, 0.66-2.32), respectively. Among *H.pylori* seropositive controls, the ORs for gastric atrophy were 1.12 (95% CI, 0.73-1.71) and 0.99 (95% CI, 0.53-1.87), respectively.

Table 1 shows the distribution of the ACE genotype in subjects as a whole and in H. pylori seropositive subjects with gastric atrophy. Among all subjects, the ORs of the I/D and D/D relative to the I/I for gastric cancer were increased, but this did not achieve stastistical significance. However, compared between cases and controls with seropositive atrophy, the OR of the I/D for gastric cancer was significantly increased (OR=1.59; 95% CI, 1.02-2.48).

We found no difference in the age at diagnosis of gastric cancer between the presence and absence of the I/I genotype (a Wilcoxon test; p=0.22, Figure 1).

Discussion

In the present study, the ACE polymorphism was not associated with H. pylori seropositivity and gastric atrophy. However, the I/D genotype increased the risk of gastric cancer among *H. pylori* seropositive subjects with atrophy. We also found that ACE polymorphism did not alter the age at diagnosis of gastric cancer.

There was a considerable ethnic variation in frequency of the I allele of the ACE polymorphism; the prevalence was 40%, 46%, 52%, 66% among African American, whites, Latians, and Japanese, respectively (Haiman CA et al., 2003).There were no substantial differences in the genotype frequency of our study controls from Japanese subjects of other studies by Kowa et al (2005) and Haiman CA et al (2003).

H. pylori is the major acquired risk factor for noncardia gastric cancer (Blaser, 1999). Correa et al described a stepwise model of changes in the gastric mucosa after H. pylori infection, from the normal gastric epithelium to chronic gastritis, atrophy, intestinal metaplasia (IM), and adenocarcinoma (Correa et al., 1975). Kitahara et al (1998) reported that gastric atrophy, defined by serum pepsinogen levels, was associated with gastric cancer. Due to the absence of histological assessment of the gastric mucosa, this study examined the associations among four steps; seronegative, seropositive, seropositive atrophy, and gastric cancer. A possible association of the genotype was found only for the comparison between the last two steps.

A previous study reported that ACE influences gastric tumor progression and metastatic behavior, but not gastric cancer risk (Röcken et al., 2005). Although our study cases consisted of advanced gastric cancer patients similarly to the previous study, the ACE polymorphism was associated with the risk of developing gastric cancer among *H. pylori* infected subjects with atrophy in our subjects.

The present result indicated that ACE polymorphism may influence the gastric atrophy /metaplasia-cancer sequence. The actual biological mechanism needs to be investigated. Angiotensin, the product of the enzyme activity of ACE, may enhance other cytokine and growth factors that may be involved in neoplastic transformation. We hypothesized that in carriers of D allele which was associated with higher ACE activity (Rigat et al., 1990), persistent exposure to higher angiotensin II during an individualûs lifetime may lower the age at diagnosis. Our result was not consistent with this hypothesis.

In summary, our study showed that the polymorphism of ACE gene was associated with gastric cancer among *H. pylori* seropositive subjects with atrophy. Because the studies on the ACE polymorphism remain limited, confirmation of the association of ACE polymorphism with gastric cancer risk requires much larger studies. Since the strength of the association may depend on the studied population, studies of different ethnic groups with different genetic profiles for gastric cancer are also required.

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